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To: Siegfried Ruppert	From: Lynn Bristol
Application/Control Number: 10/678,639	Art Unit: 1643
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☐ **Urgent** ☐ **For Review** ☐ **For Comment** ☐ **For Reply** ☒ **Per Your Request**

Comments:
Mr. Ruppert,

Please find attached a copy of the You et al. reference abstract which we discussed in our telephone conversation of 8/27/07.

Thank you,
Lynn Bristol

Number of pages 2 including this page

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ogen is believed to play a role in the degradation of fibrin and various extracellular matrix proteins, taking part in physiological and pathological tissue remodeling processes including tumor invasion. Angiostatin, a potent endogenous inhibitor of angiogenesis, is generated by proteolysis of plasminogen by CTSD. The RNA levels for both plasminogen and CTSD were suppressed in the livers of DCA-treated mice. These results are consistent with the fact that DCA induces tumor formation in mouse livers. Another function of CTSD is the mediation of IFN- γ and TNF- α induced apoptosis. The decreased level of CTSD is consistent with published data that DCA suppresses apoptosis of hepatocytes in mice. Des-gamma-carboxyprothrombin, a derivative of prothrombin in the absence of Vitamin K, has been used as a tumor marker for hepatocellular carcinoma. The Pi class of GST is the most ubiquitous of the GST family and can protect cells from carcinogenic chemicals. In conclusion, we have found 7 genes involved in cell growth, cell differentiation, apoptosis and cancer formation that are affected by DCA. (This abstract does not necessarily reflect EPA policy)

#3273 Differentially Expressed Genes in Human Prostate Cancer Cells During Progression of Hormone-Refractory Growth. Dev Karan, David L. Kelly, Ming-Fong Lin, Angie Rizzino, and Surinder K. Batra. *Eppley Institute for the Research in Cancer and Allied Diseases, Omaha, NE, and University of Nebraska Medical Center, Omaha, NE.*

In spite of the high incidence and mortality rate, the genetic causes underlying the progression of prostate cancer are not well understood. This may be due to the lack of a suitable model as well as the tremendous heterogeneity in the tumor. A unique LNCaP cell model has been developed in which the later stages exhibit different responsiveness to androgen stimulation (Lin et al. *J. Biol. Chem.* 273: 5939-47, 1998). This model has provided the opportunity to study the progressive genetic changes in prostate cancer. To test the hypothesis of differential gene expression during prostate cancer progression, a microarray filter containing 1176 known genes was analyzed using cDNA probes derived from two sublines (C-33 LNCaP: androgen-responsive and C-81 LNCaP: androgen-unresponsive) of LNCaP cell model. Twelve genes including, c-myc, c-myc purin-binding transcription factor/nucleoside diphosphate kinase B (NDKB), transforming protein rhoA H12, activator 1 37-kDa subunit, endonuclease III homolog 1, macrophage inhibitory cytokine 1 (MIC 1), migration/ glycosylation inhibitory factor (MIF/GIF), elongation factor 1 alpha, 40S ribosomal protein S16, 60S ribosomal protein L10/tumor suppressor QM, 40S ribosomal protein S5, 60S ribosomal protein L32, exhibited differences in their expression pattern. Two of these genes (MIC1 and MIF/GIF) were analyzed by RT-PCR, and one gene (MIF/GIF) showed a correlation with microarray analysis. Furthermore, differential expression of MIF/GIF was confirmed by northern blotting, showing a higher level of expression in androgen-unresponsive as compared to androgen-responsive cells. Therefore, it suggests that an enhanced expression of MIF/GIF gene in androgen-unresponsive cells may be associated with the transition from androgen-responsive to androgen-unresponsive prostate cancer. (This work was supported in part by the Nebraska Research Initiative).

#3274 Identification of a Novel Gene Induced by Virus-mediated Transfer of Wild-Type AXIN1 by Means of cDNA Microarray. Y. Nakamura, T. Tsunoda, H. Okabe, Y. Lin, T. Katoh, N. Miwa, M. Fujita, S. Satoh, Y. Furukawa, and H. Ishiguro. *Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.*

Axin plays an essential role in the degradation of β -catenin in concert with APC and Glycogen synthase kinase-3 β (GSK-3 β) upon Wnt signaling. Adenovirus mediated gene transfer of wild-type AXIN1 resulted in reduction of nuclear β -catenin level and decreased activity of β -catenin/Tcf4 dependent transcription, leading to an induction of apoptosis in HCC and colon cancer cells with accumulated β -catenin as a consequence of either APC, β -catenin, or AXIN1 mutation. These results implied that Axin could be an effective therapeutic molecule for a wide range of hepatocellular and colorectal cancers. To disclose genes regulated by β -catenin/Tcf4 complex and mechanisms of apoptosis induced by AXIN1, we investigated genes differentially expressed in response to the transduction of the AXIN1 gene in LoVo cells established from colon cancer. RNA extracted from cells infected with a control gene, LacZ, and RNA with the AXIN1 gene was labeled with fluorescent dye, Cy3 or Cy5, respectively. Comparison of the expression profiles by means of cDNA microarray representing 23,040 genes facilitated identification of a number of genes with altered expression. Among the genes identified, a novel human gene, termed A5900, was up-regulated in response to the gene transfer of AXIN1. Furthermore, it showed suppressed expression in 16 out of 20 colon cancer tissues. Interestingly, localization of this gene was assigned at chromosomal band 3p22 where many reports suggested the existence of multiple tumor suppressor genes for lung, kidney, and cervical cancers. Investigation of the function of genes regulated by AXIN1 will provide better understanding of human carcinogenesis associated with accumulated β -catenin and that of mechanisms of apoptosis induced by AXIN1.

#3275 Differentially Expressed Wnt Genes in Human Mesotheliomas. Liang You, Kazutsugu Uematsu, Michael J. McBrine, Frank McCormick, and David M. Jablons. *University of California San Francisco, San Francisco, CA.*

Human cancer cDNA expression array was used to study differential gene expression between non-neoplastic pleura and malignant pleural mesothelioma dissected from patients with malignant pleural mesothelioma. This cancer array is

designed for high-throughput analysis of molecular events involved in tumor development. Matched mesothelioma and non-neoplastic pleura from five patients were studied for their expression of these genes. In this study, down-regulation of Frizzled-related protein (FRP2), a Wnt signal antagonist, has been found in three out of the five mesotheliomas when compared to their non-neoplastic counterparts. We are currently making the antibody against FRP2 and will be analyzing the protein expression in these tumors. In addition, Dishevelled (Dvl), a Wnt signal transducer, is overexpressed in four of the five mesotheliomas tested. Phosphorylated Dvl protein was only noticed in the mesotheliomas. No mutations were detected in the exon 3 of b-catenin gene. Functional studies are currently underway in our laboratory using these tumor cells. Our data suggested the possible involvement of Wnt signal transduction pathway in mesothelioma development. With further studies, the data from these arrays may help us to better understand Wnt pathway in mesotheliomas and may lead to discovery of new therapeutic targets.

#3276 DNA Microarray Analysis of Changes in Gene Expression Induced by over Expression of Wild Type Thioredoxin-1 or a REDOX Inactive Mutant Thioredoxin-1 in MCF-7 Human Breast Cancer Cells. B. Husbeck, A. Coon, and G. Powis. *University of Arizona, Tucson, AZ.*

Thioredoxin-1 (Trx-1) is a redox protein found at increased levels in a number of human primary tumors where it is associated with increased proliferation and decreased apoptosis. Transfection of cancer cells with Trx-1 stimulates cell growth and inhibits both spontaneous and drug induced apoptosis while a redox inactive mutant Trx-1 Cys32 \rightarrow Ser/Cys35 \rightarrow Ser (SerB) inhibits cell growth and potentiates apoptosis. We have used DNA microarrays of 520 cancer-related genes to examine the effects of wild-type Trx-1 and SerB overexpression in MCF-7 breast cancer cells. Our goal was to identify genes increased in the wild-type Trx-1 transfectants and decreased in the SerB transfectants, or *vice versa*. Microarray analysis identified 15 candidate genes whose expression was altered by Trx-1 or SerB transfection. The expression of both cytochrome P450 1B1 (Cyp1B1) and glutathione-S-transferase M4 (GST M4) was increased in the Trx-1 transfectants and decreased in the SerB transfectants. The expression pattern for both of these genes has been confirmed by Northern and Western analysis. Cyp1B1 message is increased five-fold in Trx-1 transfectants and decreased two-fold in SerB transfectants relative to control. Microarray analysis also revealed an increase in the expression of thioredoxin peroxidase-1 (PRDX1, proliferation associated gene) by cells transfecting with wild-type Trx-1. Northern analysis has confirmed a two-fold increase in PRDX1. No change was observed in the SerB transfectants. Support by CA 48725 and CA77204.

#3277 Differential Gene Expression Patterns Associated with the *In Vitro* Malignant Transformation of Human Ovarian Epithelial Cells and Chemopreventive Treatment with Fenretinide. Troy D. Querec, Briana N. Gruver, Photis C. Patriotis, Radka S. Stoyanova, Andrey E. Frolov, Paul F. Engstrom, Andrew K. Godwin, Truman R. Brown, and Christos Patriotis. *Fox Chase Cancer Center, Philadelphia, PA.*

The differential response to chemopreventive treatment of Human Ovarian Surface Epithelial (HOSE) cells at different stages of malignancy was examined with regard to cell viability and changes in the patterns of gene expression. Primary cells were obtained from ovaries prophylactically removed from ovarian cancer-prone individuals. These primary cells were transduced with the SV40 large T-antigen and established into a series of HOSE cell lines with growth characteristics ranging from normal to overtly malignant. Individual HOSE cell lines with different malignant potentials were treated with the synthetic retinoid acid derivative Fenretinide, and the effect on cell viability was determined by MTT assays and flow cytometric analysis. The differential response of the cells to this treatment was also examined by monitoring the changes in the expression patterns of $\sim 1,200$ genes. This was carried out using the Atlas 1.2 Human Cancer filter arrays from CLONTECH with total RNA extracted from treated and untreated cells. The data were analyzed with a custom image analysis software, *ArrayExplorer*, developed in the laboratory. This software facilitated the processing of the array images and the extraction of gene expression intensities, which were further analyzed by multivariate statistical analysis techniques. Preliminary analysis of gene array expression data identified gene clusters that undergo coordinated changes both during the *in vitro* malignant transformation and in response to chemopreventive treatment with Fenretinide. Such gene clusters should provide important insights with regard to the molecular genetic mechanisms associated with ovarian epithelial oncogenesis and may serve as potential biomarkers for the efficient response to chemopreventive treatment.

#3278 Use of cDNA Microarray to Analyze Gene Expression Pattern in Nickel Treated Human Lung Cells. Yuk Sing Robert Cheng, Robert M. Bare, Takashi Takahashi, Akira Masuda, Lucy M. Anderson, and Kazimierz S. Kasprzak. *Cellular Pathogenesis and Metals Sections, National Cancer Institute at Frederick, Frederick, MD, and Division of Molecular Oncology, Aichi Cancer Center Research Institute, Nagoya, Japan.*

Nickel and certain nickel compounds are carcinogenic for the lung, nose and perinasal sinuses in humans. The mechanism of their carcinogenic effects is unknown; epigenetic effects are suspected. To investigate systematically possible changes in gene expression associated with nickel exposure of lung cells, we incubated immortalized human lung type II cells (HPL 1D) with a series of